[normal] karyotype through prolonged culture, the karyotype being normal in the sense that all chromosomes characteristic of the species are present and not visibly altered, (iii) maintains the potential to differentiate [to] into derivatives of endoderm, mesoderm, and ectoderm tissues throughout the culture, and (iv) will differentiate in the presence of human leukemia inhibitory factor alone, but will not differentiate when cultured on a fibroblast feeder layer alone.

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3. (Amended) A purified preparation of primate embryonic stem cells wherein the cells are negative for the SSEA-1 marker, positive for the SSEA-3 marker, positive for the SSEA-4 marker, express alkaline phosphatase activity, are pluripotent, are capable of proliferating in an *in vitro* culture for over one year while retaining pluripotent capability, and have normal karyotype in the sense that all chromosomes characteristic of the species are present and not visibly altered.

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9. (Amended) A method of isolating a primate embryonic stem cell line, comprising the steps of:

(a) isolating a primate blastocyst;

(b) isolating cells from the inner cell mass of the [blastocyte] blastocyst of (a);

(c) plating the inner cell mass cells on embryonic fibroblasts, wherein inner cell mass-derived cell masses are formed;

(d) dissociating the mass into dissociated cells;

(e) replating the dissociated cells on embryonic feeder cells;

(f) selecting colonies with compact morphologies and cells with high nucleus to cytoplasm ratios and prominent nucleoli; and

(g) culturing the cells of the selected colonies.

10. (Amended) A method as claimed in claim 9 further comprising maintaining the isolated cells [or] on a fibroblast feeder layer to prevent differentiation.

Remarks

This Amendment is made in response to the Office Action dated January 17, 1996 in the file of this patent application. In that Office Action,

all of the claims were objected to for reasons of indefiniteness, and all the pending claims were rejected for obviousness on the prior art. All the claims rejected for indefiniteness have been amended above and arguments as to the prior art rejections are made herein. On this basis, reconsideration of the merits of this patent application is respectfully requested.

In the Office Action, the Examiner first rejected all of the claims for indefiniteness under 35 U.S.C. §112, second paragraph. The Examiner pointed out several wording informalities in the claims, all of which have been addressed above. The language objected to in claim 1 has been amended and the clear clerical inaccuracies in claims 1, 9 and 10 pointed out by the Examiner have been corrected. The applicant disagrees with one point of objection by the Examiner, however. It is submitted that the phrase "normal karyotype" is one having a definite meaning in the art and that is well understood and accepted scientifically. See, for example, the Bongso et al. paper from *Human Reproduction*, which uses that term on page 2114. The term is also defined by the applicant on page 22, lines 5-8 of the specification. However, to avoid any dispute on this relatively ancillary issue, the applicant has added language to both claims 1 and 8 which defines normal karyotype. It is hoped that this amendment will satisfy the Examiner on this point.

The first rejection on the merits is under 35 U.S.C. §102(a) over a document identified as "Nation/World." That document is actually a copy of a page from the November 4, 1994, issue of the Milwaukee Journal newspaper. The page includes an article describing, in very brief layman's terms, the results of the work of the applicant here. The James Thomson identified in the newspaper article is the inventor here, a professor at the University of Wisconsin-Madison. The John Hearn identified in the article is a technical assistant who was then working under the direction of Dr. Thomson.

This rejection is traversed for two reasons. First, for a rejection to be viable under 35 U.S.C. §102, the asserted reference must meet all of the limitations of the claimed invention. Clearly the newspaper report does not disclose the special features of the primate embryonic cell preparations claimed in claim 1 and 3, and just as clearly it does not disclose or teach the

method of claim 9. The conduct of experimentation or laboratory work is not required to reach this conclusion. The article simply teaches nothing of the subject matter in any of the claims; it is conclusory and superficial only. No one of any level of skill in the art could figure out from the contents of that article exactly what characteristics the reported stem cells had, or how the stem cell cultures were made. Thus, it is asserted that the document is not a proper §102 reference, since it is simply nowhere close to enabling. A non-enabling disclosure cannot support a rejection under §102, any subsection.

The second reason for the traversal of this rejection is that the work is not the invention of another. The work described in the article is the work of the applicant here. It would be a triumph of form over substance if the applicant here is required to submit a declaration to establish that he did actually perform the work reported as being done by him, before the publication occurred. The newspaper could not logically report on the work before Dr. Thomson did the work. Section 102(a) requires the publication occur before the invention by the applicant, and it is a logic impossibility for the inventor here to have invented after doing the newspaper article, since the article itself report he had already done it.

Obviously, the applicant can "swear ahead" of this newspaper article under rule §1.131, and if required to do so by the Examiner, will do so. However, it is asserted that this should not be required, since the newspaper article cannot support a proper §102 rejection in any event.

The next rejection imposed by the Examiner is also under 35 U.S.C. §102(a), or alternatively under §103, over either of two publications by Bongso et al. The examiner notes correctly that the ES cells reported by Bongso et al. were alkaline phosphatase positive and were derived from inner cell masses of primate embryos. The Examiner then notes that the Patent Office has no laboratories to test the respective cells, and based on that, the Examiner asserts that since there are some traits in common between the cells of Bongso et al. and those of the applicant here, that the burden is placed on the applicant to demonstrate that there is a non-obvious difference between the cells.

The applicant is actually at a disadvantage here as well. The applicant also does not have access to the cells reported by Bongso et al., who are from Singapore and able to do experiments that would not be

approved in the United States for NIH-funded researchers. However, the applicant proposes to surmount that difficulty by relying on a clearly distinguishing and unobvious feature of the applicants' cultures, as distinguished from the cultures of Bongso et al. That feature, now clearly claimed in both claims 1 and 3, is longevity in culture. Both claims 1 and 3 now recite the limitation that the ES cell cultures can be maintained in *in vitro* culture for over one year while retaining pluripotent capability. Bongso et al. candidly reported that their cultures simply could not do this.

In the Bongso et al. *Human Reproduction* paper, the authors explicitly reported that, "After second subculture, the cells differentiated into fibroblasts or died." (Bongso et al. page 2114, right-hand column) The first subculture was reported to be after 10-14 days (page 2112). The second subculture was reported to be after 8-10 days (also page 2112). Thus the cells were explicitly reported to have a lifetime as stem cells, without freezing, of no longer than 24 days.

By contrast, the stem cell cultures of the applicant here are quite capable of cultivation *in vitro* for time periods in excess of one year while retaining pluripotent capabilities. In fact, continued cultivation for one year is considered by many in the art to be evidence of immortality (specification page 22). The applicant explicitly reports that he subcultures the embryonic cell lines every 1-2 weeks, meaning a minimum of 26 subcultures in a year (specification page 16). Even after that long time period and extensive subculturing, the primate ES cells retain pluripotency, as determined by the ability to differentiate into any of the three primary cell types *in vivo* as verified by test with SCID mice (specification pages 20-21).

This ability to have longevity is quite obviously an highly essential characteristic of a practical system for stem cell culture. Only by cultivation of continuous culture of standard genetic type can experiments with cells derived from the ES culture be performed with common conditions. Only with long-term controlled cultures can the prospect of developing systems for tissue differentiation be studied. In short, while the results of Bongso et al. might be tantalizing, their failure to achieve longevity in the their cultured ES cells makes their work a laboratory curiosity rather than the basis of a practical usable system.

Thus, it is submitted that there is a very clear difference between the cultures recited in claims 1 and 3 and those reported by Bongso et al. To

ascertain that difference, no laboratory tests are required, because the difference is clearly reflected in the respective reported results. Thus, a rejection under 35 U.S.C. §102 is believed overcome.

It is similarly submitted that a rejection under 35 U.S.C. §103 is also overcome by reliance on this limitation. It is submitted that the difference between the cultures of the applicant and those of Bongso et al. is a non-obvious one. Bongso et al. teaches, in fact, that ES primate cultures cannot subcultured more than twice. Bongso et al. thus teaches away from this feature of the applicant's ES cultures. The applicant has demonstrated practical immortality for his primate ES lines. This is a dramatic difference that is the difference between practicality and impracticality. Therefore, it is submitted that claims 1 and 3, which both now recite the longevity limitation, are in no event obvious, within the meaning of 35 U.S.C. §103, over the publications by Bongso et al.

The final rejection imposed by the Examiner is to the method claims 9-11 for obviousness of Piedrahata et al., which describes the creation of ES lines in murine, porcine and ovine systems. From this, the Examiner argues that the functioning of the applicant's method in primates was predictable with reasonable certainty. The applicant disagrees.

For support, the applicant turns to the publication which the Examiner has asserted is closest to the work of the applicant here, the Bongso et al. publication in *Human Reproduction*. On page 2110, Bongso et al. note, in passing, that, "However, Notarianni *et al.* (1990) pointed out that the methods devised for ES cell production for one species may not be directly applicable to another species..."

The applicant asserts that, in this instance, Bongso et al. were right. In this field of technology, empiricism rules. The methods from one class of animal might or might not be obvious to try in another animal, but the persons of high skill in the art still do not believe that they can predict whether methods worked out in one species will or will not work in another distantly-related species. Bongso et al. say as much. This is a clear situation of "obvious to try," since one might be motivated from the cited reference to try this general approach, but this is not a situation in which is was obvious from the reference that the claimed method would work. In essence, the applicant argues that work from mice or sheep simply does not provide sufficient guidance, in this art, to demonstrate a reasonable

expectation of success in primates. Accordingly, it is submitted that the *prima facie* case for obviousness is not made.

The Examiner also notes that even if a *prima facie* case for obviousness has been made, it would be overcome by a showing of unexpected results. It is submitted here that the longevity of the applicant's cultures is such an unexpected result. If one considers the art as a whole, including the teaching of Bongso et al., one of skill in the art would be lead to believe that long-term primate ES cultures could not be made by this method. The applicant has demonstrated otherwise. This is an unexpected result sufficient to overcome this rejection.

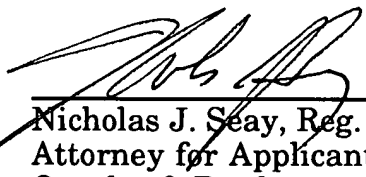
Accordingly, reconsideration of this rejection is respectfully requested.

Out of an exercise of a duty of candor, the applicant also enclosed herewith a copy U.S. Patent 5,453,357, not yet issued when the applicant files his IDS. The actual work reported in the patent appears to be limited to murine cultures.

The applicant also wishes to make the Examiner aware of a related application, Serial No. 08/591,246, filed January 18, 1996 by the inventor here.

Wherefore, the Examiner is respectfully requested to revisit the merits of the specification and claims of this patent application. An early and favorable reply is solicited.

Respectfully submitted,



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